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FORMULATION OF SUNGKAI LEAF ETHANOL EXTRACT POWDER (*Peronema canescens* Jack.) AND ITS ANTIOXIDANT TEST

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K a t a K u n c i

Sungkai leaf (Peronema canescens Jack.)
antioxidant
instant powder
DPPH

P e n u l i s K o r e s p o n d e n s i

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A B S T R A C T

Sungkai leaves (Peronema canescens Jack.) is one of the medicinal plants that have antioxidant activity. This study aims to formulate sungkai leaf ethanol extract in the form of instant powder as a health supplement and test the antioxidant activity of instant powder preparations. Making dry extract of sungkai leaves using freeze dryer and antioxidant activity test using DPPH method at a maximum absorption wavelength of 520 nm. The results of instant powder formulation on organoleptis examination instant powder is in the form of fine powder, white with green grains, distinctive smell and slightly sweet taste while the brewed instant powder is in the form of a green liquid, distinctive smell and slightly sweet taste, good flow properties, flow speed 6.63 g / s, resting angle 29.45^{o}, soluble time 110 seconds, water insoluble part 1.528%, moisture content 5.12%, ash content 0.29% and pH 5.59. From all examinations have met the requirements of good preparations. IC values of$_{50}$ ethanol extract and instant powder were 73.76 ppm and 662.69 ppm respectively. So it can be concluded that sungkai leaf ethanol extract can be formulated as instant powder and sungkai leaf ethanol extract has strong antioxidant activity, while sungkai leaf instant powder has very weak antioxidant activity.

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INTRODUCTION

Sungkai (Peronema canescens Jack.) is a member of the Lamiaceae family and is often referred to as sekai, kurus sungkai, ki sabrang, or teak sabrang. Until now, the Dayak tribe in East Kalimantan still maintains the tradition of utilizing surrounding plants for medicine and health care, such as the sungkai plant (Peronema canescens Jack). Jambi people traditionally use a decoction of sungkai leaves to treat malaria, child seizures, postpartum, fever, and poisoning.(Rahman et al., 2021).

In sungkai leaves, it finds phenolics, tannins, alkaloids, steroids, saponins, and flavonoids. (Meylisa Pratami Br Sinaga et al., 2022). One of the natural phenolic compounds that has the potential to function as antioxidants is flavonoids. Flavonoids do this by stopping reaction oxidation, the process by which hydrogen atoms are donated to bind free radicals and other active oxygen. (Sigit Cahyo Hardiansyah & Pheby Oktriani, 2021).

Molecules known as antioxidants can prevent oxidation of molecules that can produce free radicals.(Pamunuwa & Atapattu, 2023). A compound or molecule that has one or more unpaired electrons in its outer orbitals may be referred to as a free radical. (Amin et al., 2019). Radicals with high reactivity will form a chain reaction, in one formation can cause abnormal compounds and can damage important cells in the body (Leifert et al, 2020)

With the effects of the pandemic, Indonesian people are more likely to consume sungkai leaves as a drink that can increase endurance, namely by drinking fresh sungkai leaf boiled water. However, the resulting cooking water has a bitter taste and aroma that is less preferred, so it needs to be processed so that it is easy to consume. For example, it can be made in the form of herbal drinks such as tea or instant powder.

Based on the explanation above, researchers are interested in researching instant powder formulations from sungkai leaf ethanol extract (Peronema canescens Jack) as a health supplement which in its use is brewed into a beverage and tested its antioxidant activity using the DPPH test method.

METHODOLOGY

Instrument and Materials

Instrument

The tools used are Spectrophotometers UV-Vis (T92), moisture balance (Ohaus, USA), furnace (Wise term), watch glass, evaporator cup, crutch, measuring cup (Pyrex®), Cup glass (Pyrex®), funnel, digital scale (Ohaus, USA), A set of instrument rotary evaporator (Hettich centrifuges), freeze dryer, pH meter, aluminum foil, graph paper, oven (Memert), Sieve (Fritsch, Germany), mortar and pestle, measuring pipette (Pyrex®), desiccator, blender (Philips, Belanda), stopwatch.

Material

The ingredients used in this study were fresh sungkai leaves, maltodextrin, sucrose, ethanol 70%, aqua dest, methanol p.a (Merck), DPPH (Smartlab), chloroform (Merck), Mg, HCl (P), FeCl 3, norite, H_2SO_4 (Merck), anhydrous acetic acid (Merck), chloroform ammonia, Mayer reagent.

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Making Sungkai Leaf Thick Extract

Sungkai leaf powder is extracted by maceration. 500 grams of jackfruit leaf powder is weighed, put in a vessel, 70% ethanol is added, and allowed to stand for three days while stirring occasionally. After three days of soaking, it is filtered with filter paper to extract the fiber, then evaporated with a rotary evaporator to produce a viscous extract. (Harbone, 1987).

Examination of Sungkai Leaf Viscous Extract

1. Organoleptic Examination
   Done visually by observing the shape, color, taste, and smell of the extract.
2. Determination of Extract Yield
   The yield of the extract is measured in the following way:

$$\% \text{ Rendemen} = \frac{\textit{Weight of extract obtained}}{\textit{Fresh extract weight}} \text{x} 100\%$$

3. Phytochemical Test
   One gram of sungkai leaf extract is put into a test tube along with five milliliters of aquadest and five milliliters of chloroform. Then shake and leave until two layers of water and chloroform are formed. After that, it is separated. (Herborne, 1987).

   a. Flavonoid Test
      After taking a layer of water 1-2 drops, drip on the drip plate and add Mg and HCl (P) powder. The red color indicates the presence of flavonoids.

   b. Phenolic Test
      1-2 drops of water are taken, dripped on a drip plate, then FeCl3 reagent is added. The blue color indicates phenolic content.

   c. Saponin Test
      A layer of water is taken, and shaken vigorously in a test tube, permanent foam formation (±15 minutes) indicates the presence of saponins.

   d. Test Terpenoids and Steroids
      After norite is added to a small amount of chloroform, the coating is put into a drip pipette with a cotton tip, and then inserted into a drip plate. During the mongering process, 2 drops of concentrated H2SO4 and anhydrous acetic acid are added. Blue or green indicates the presence of steroids, while red indicates the presence of terpenoids.

   e. Alkaloid Test
      A small layer of chloroform is taken, 10 mL of ammonia chloroform 0.05 N is added, stirred slowly is added a few drops of H_2SO_4 2N, then shaken slowly, left to separate. A layer of acid is inserted into a test tube, and a few drops of Mayer

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reagent are added, the positive reaction of alkaloids is characterized by the presence of white mist to white lumps.

4. Solubility Check
5. Drying Shrinkage Inspection
   The viscous extract weighed 1 g and was put into a steamer dish that had previously been heated in the oven and weighed. Then the steamer dish containing the extract is put into the oven at 105^{o}C for 1 hour and then cooled in a desiccator and weighed until a fixed weight is obtained (Depkes RI 1979).

$$\% \text{ Shrinkage Drying} = \frac{(B-A)-(C-A)}{(B-A)} \text{ x } 100\%$$

6. Ash Content Check
   The extract weighed 2 g and then put into the previously incandescent porcelain crutches. Krus are put into Furnes at a temperature of 600^{o}C for 4 hours until the charcoal runs out which is marked with gray. After cooling, weighed (Depkes RI, 1979).

$$\% \text{ Ash content} = \frac{C-A}{B-A} \text{ x } 100\%$$

7. pH Test

Making Dried Extract of Sungkai Leaves

Making dry extract of sungkai leaves is done after obtaining a thick liquid of sungkai leaf extract, the liquid is dried using a freezer dryer at a temperature of (-90^{o} C) for 24 hours. The dried extract of sungkai leaves is then crushed by grinding.

Table 1. Sungkai Leaf Instant Powder Formula

Material Name	Formula (%)
Dry Extract Sungkai Leaves	3
Sucrose	25
Maltodextrin	100

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Instant Powder Formulation of Sungkai Leaf Ethanol Extract

Instant powder of sungkai leaf extract is made by weighing all ingredients, then dry extract of sungkai leaves added with sucrose and maltodextrin, crushed until instant powder is formed, and sifted with a mesh sieve 60.

Evaluation of Sungkai Leaf Instant Powder

1. Organoleptic Examination
   - Instant Powder Organoleptic Test
     Carried out visually by observing the shape, color, taste, and smell of instant powder preparations.
   - Brewed Instant Powder Organoleptic Test
     Done by weighing 5 grams of instant powder preparation then dissolving it in 50 ml of water, then observing the shape, color, taste, and smell of the brewed instant powder (observation made by 5 people)
2. Break Angle
   A total of 30 grams of powder is inserted into the funnel where the bottom hole is closed and then flattened on the surface of the funnel that is given a base. The bottom lid of the funnel is opened so that the powder can flow onto the table that has been lined with graph paper. The angle of rest is done by measuring the height and diameter of the base of the formed powder pile and calculating the time it takes for the powder to flow using a stopwatch. (Cartensen dkk, 1997).

$$\text{tg } \alpha = \frac{h}{r}$$

   Description of the formula :
   h = cone height (cm)

   r = radius (cm)

   α = Silent Angle

$$\text{Flow Speed} = \frac{\text{Powder weight(g)}}{\text{Powder flow time (second)}}$$

3. Water Content
   Measurement of moisture content using a moisture balance tool. A total of 1 gram of powder is placed into the tool above the pan. The tool will automatically measure the water content at a temperature of 105^{o}C for 15 minutes and the results will automatically appear on the monitor screen expressed in %MC.
4. Ash content
   2 grams of powder is inserted into the previously incandescent porcelain crutches. The crucibles are cooled in a desiccator and put into furnaces at 600^{o}C for 4 hours, until the charcoal runs out which is marked with a gray color. After cooling, weighed (Depkes RI,1979).

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$$\% \text{ Ash content} = \frac{C-A}{B-A} \times 100\%$$

Information :
A = Empty krus weight (g)
B = Krus weight + sample before teaching (g)
C = Crutch weight + sample after teaching (g)

5. Water Discharge Time
   Weighing 5 grams of sample then dissolved in 50 ml of water then stirred until homogeneous recorded how long the sample took until completely dissolved in water (Widiatmoko dan Hartono, 1993)
6. Water Insoluble Parts
   Weigh 5 grams of sample then put into a 500 ml cup glass, add 200 ml of water then stir until dissolved. After harvesting into filter paper that has been dried in the oven and known weight. Cup cups and filter paper are rinsed with aquades until the residue is obtained on the filter paper. Dried filter paper in the oven at 105^{o}C for 2 hours, cool the filter paper in a desiccator, and weigh (SNI 01-2891-1992).

$$\text{Water Insoluble Parts} = \frac{W2-W1}{W3} \times 100\%$$

Information :
W1 = Weight of blank filter paper (g)
W2 = The weight of the filter paper contains water-insoluble parts (g)
W3 = Sample weight (g)

7. pH Test
   pH checks are carried out using a pH meter. The sample was weighed as much as 1 gram and dissolved in 10 mL aquadest. Then the electrode is dipped in solution and left with a digital number indicating pH to a constant position (Departemen Kesehatan Republik Indonesia, 1979).

Determination of Antioxidant Activity by DPPH Method
Manufacture of DPPH Master Solution35 μg/ml

A total of 10 mg of DPPH powder is put into a 100 mL measuring flask and then methanol p.a until the limit mark. Then pipette as much as 35 mL of DPPH solution is inserted into a measuring flask of 100 mL, added m ethanol p.a until the limit mark until a solution with a concentration of 35 µg / mL is obtained.

DPPH Maximum Absorption Wavelength Test 35 μg/ml

A pipette of 4 ml of freshly made DPPH 35 μg/mL solution is put into the vial, added 2 ml of a mixture of methanol p.a and aquadest (1:1), close the vial and leave for 30 minutes in

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a dark place. Absorption is measured with a UV-Vis spectrophotometer at wavelengths of 400-800 nm.

Test of Antioxidant Activity of Sungkai Leaf Ethanol Extract with DPPH Method

Weighing 25 mg of sungkai leaf extract, then dissolved in methanol p.a in a 25 mL measuring flask to the limit mark with a concentration of 1000 μg / mL as the parent solution. The mother solution is pipetted as much as (0.4; 0.5; 0.6; 0.7; 0.8) ml and put into a 10 ml measuring flask. Then a mixture of methanol p.a and aquadest (1:1) is added until the limit mark, until a concentration is obtained (40; 50; 60; 70; and 80 μg/mL).

Add 2 mL of test solution of various concentrations that have been made, and add 4 mL of DPPH solution. Shaken until homogeneous, then left for 30 minutes in a dark place, then measured absorption using a UV-Vis Spectrophotometer at a maximum absorption wavelength of 520 nm. Then the percent value of inhibition and $IC_{50.}$

Antioxidant Activity Test of Instant Powder of Sungkai Leaf Ethanol Extract with DPPH Method

Weighing 50 mg of instant powder of beech leaves, then dissolved with methanol p.a in a 25 mL measuring flask to the limit mark so that a concentration of 2000 μg / mL was obtained as the parent solution. The mother solution is pipettes (0.75; 1.5; 2.25; 3; 3.75) ml and put into a 10 ml measuring flask, then a mixture of methanol p.a and aquadest (1:1) is added to the limit mark, until a concentration is obtained (150; 300; 450; 600; 750μg/mL).

Pipetted 2 mL of various concentrations of the the test solution and put into the vial, add 4 mL of DPPH solution 35 μg/mL. Shaken until homogeneous, then left for 30 minutes in a dark place, then measured absorption using a UV-Vis Spectrophotometer at a maximum absorption wavelength of 520 nm. Then the percent value of inhibition and $IC_{50.}$

RESULT AND DISCUSSION

This study was conducted to formulate ethanol extract of sungkai leaves (Peronema canescens Jack.) into health supplements in the form of instant powder preparations and see the antioxidant activity in instant powder preparations. Sungkkai leaf samples were taken from the Lubuk Alung area, Padang Pariaman, West Sumatra. The sample was identified first in the Herbarium of the Department of Biology FMIPA Andalas University. Based on the identification results showed that the sungkai leaves used had the Latin name Peronema canescens Jack. from the Lamiaceae family.

Furthermore, an examination of the ethanol extract of sungkai leaves was carried out, on organoleptic examination, sungkai leaf extract was obtained in the form of a thick green liquid with a thick green color, with a bitter taste, and a distinctive smell. In determining the yield of extract, 13.25% was obtained. Yield determination aims to determine the ratio of the

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amount of extract obtained from a material to the initial weight of the simplistic material. Examination of the solubility of the extract against water and 70% ethanol where the sungkai leaf extract is soluble in water and quite difficult to dissolve in 70% ethanol.

Phytochemical examination aims to determine the presence of secondary metabolites contained in the sample. It was found that the ethanol extract of sungkai leaves positively contained flavonoids, phenolics, saponins, steroids, and alkaloids. According to Pindan, et al (2020), sungkai leaves have flavonoid and phenolic compounds and these compounds are one of the compounds that have antioxidant activity. The determination of drying shrinkage aims to provide a maximum limit on the number of compounds lost in the heating process, not only water but also other evaporating compounds. (Ministry of Health RI, 2000). The results of the determination of the drying shrinkage of sungkai leaf ethanol extract were obtained at 9.79%, which shows that the drying shrinkage of sungkai leaf ethanol extract meets the requirements which is <10%.

Determination of ash content aims to determine the content of internal and external inorganic minerals left in the sample from the initial process until the formation of the extract (Ministry of Health RI, 2000). The results of determining the ash content obtained a value of 5.74%, the results showed that the ash content of sungkai leaf ethanol extract used was not greater than the established standard of <8% (Ministry of Health RI., 2000). The results of the pH examination of the extract obtained a value of 5.24.

The formula used in the manufacture of instant powder consists of active substances, sweeteners and fillers. The active substance used is a dry extract of sungkai leaves (Peronema canescens Jack.). The concentration of the active substance used is based on immunostimulant research of ethanol extract of sungkai leaves (Peronema canescens Jack.) in male white mice with clearance methods that have been converted to human doses. Sucrose is used as a sweetener and maltodextrin is used as a filler. The addition of maltodextrin aims to coat the flavor components, increase the volume, speed up the drying process, prevent damage to materials due to heat, and increase the solubility of powdered drinks (Husni et al., 2020). Instant powder preparation can be seen in Figure 1.

Figure 1. Instant Powder Preparation

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Furthermore, evaluation was carried out on instant powder preparations, which included organoleptic examination, resting angle, flow speed, dissolving time, water insolubility, ash content, water content, pH, and antioxidant activity tests. The results of the evaluation of the instant powder of sungkai leaves can be seen in Table 2.

Table 2. Results of Instant Powder Evaluation of Sungkai Leaves

Evaluation	Observation
Instant powder organoleptis	
Shape	Fine powder
Color	White there are green grains
Smell	Distinctive
Taste	Kinda sweet
Steeping instant powder organoleptic	
Shape	Liquid
Color	Green
Smell	Distinctive
Taste	Kinda sweet
Angle of rest	29,45±1,487
Flow speed	6,635±0,477
Water content	5,12±0,254
Ash content	0,29%
Late time	110 ±4,58
Water insoluble parts	1,528%
pH	5,59

Organoleptical examination of the preparation aims to see the physical appearance of the preparation including shape, color, smell, and taste. From the observations, instant powder was obtained in the form of a fine powder of white color with green grains smelling distinctive and having a slightly sweet taste. Then the results of organoleptis observations of instant powder brewed conducted by 5 panelists obtained steeping results in the form of a green liquid with a distinctive smell of sungkai leaves and a slightly sweet taste.

The resting angle is the angle formed from free-flowing powder on the funnel against a flat plane. A powder is said to have a good resting angle when it falls into the value range of 25-30^o, the smaller the resting angle value, the better the flow properties of a powder. The inspection result of the resting angle of sungkai leaf instant powder is 29.45^o, this shows that the sungkai leaf instant powder has met the requirements.

Checking the instant powder flow speed of sungkai leaves aims to determine the powder flow speed, whether good or not. A good flow speed requirement is 4-10 g/s

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(Assalam, 2022). The results obtained from testing the instant powder flow speed of sungkai leaves are 6.635 g / second which meets the requirements of a good powder flow time.

Moisture content check of instant powder of sungkai leaves is carried out using a moisture balance tool. From the observations, %MC is 5.12%, the moisture content requirement has met SNI 01-3722-1995, namely the content in instant powder is 3-7%. Examination of ash content on instant powder of sungkai leaves obtained a result of 0.29%. Based on SNI quality standards (01-0432-1996) the value of the ash content of instant powder drinks is not more than 1.5%, so that the results of the inspection of the ash content of instant powder leaves meet the specified requirements.

A powder dissolution time check is performed to see how long it takes for the preparation to dissolve completely in water. From the observations, it was obtained that the time dissolved in water is 110 seconds and meets the requirements of (BPOM, 2014) which is no more than 5 minutes. Examination of water-insoluble parts is done to see how many parts cannot dissolve in water. From the observations, the insoluble part of water was obtained by 1.128%.

Checking the pH of the preparation needs to be done to determine the homogeneity of the acid and base components of the instant powder, if the preparation is too acidic it can irritate the stomach and if the preparation is too alkaline it will cause the taste of the preparation to become bitter and unpleasant. The pH of instant powder of sungkai leaves was obtained at 5.59 which shows instant powder has a fairly good acidity level so it is safe for consumption on an empty stomach.

Determination of Antioxidant Activity

Determination of antioxidant activity of samples using DPPH method (1,1-Diphenyl-2-picrylhydrazil). Compounds that have antioxidant activity will react with DPPH indicated by a change in color from violet violet to yellow due to the donation of hydrogen atoms from antioxidants to DPPH (Pamunuwa & Atapattu, 2023).

The magnitude of antioxidant activity is indicated by an IC$_{50}$ value, which is the concentration of the sample solution that provides 50% inhibition of DPPH free radicals. The maximum absorption wavelength measurement of DPPH of sungkai leaf ethanol extract is 520 nm at a concentration of 35 μg/mL with an absorption of 0.451.

In antioxidant testing, ethanol extract of sungkai leaves has strong antioxidant activity and in instant powder, sungkai leaves have very weak antioxidant activity. The results of determining the antioxidant activity of sungkai leaf extract can be seen in Table 3.

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Table 3. Results of Determination of Antioxidant Activity of Sungkai Leaf Extract

Extract concentration	Abs control	Abs extract + DPPH	Inhibition (%)	IC_{50}
40	0,451	0,352	21,95	73,76
50		0,320	29,04	
60		0,275	39,02	
70		0,240	46,78	
80		0,202	55,21	

Value IC_{50} From ethanol extract obtained 73,76 µg/mL, According to research that has been conducted by (Fitria, 2021) Sungkai leaf ethanol extract has value IC_{50} sebesar 55,47 µg/mL, yang Shows that sungkai leaf extract has strong antioxidant activity. This is different from the results obtained probably due to differences in the content of secondary metabolites in plants that depend on environmental factors, plant factors themselves and the extraction method used. This is also supported by the statement of Fadilaturrahmah, et al (2021) which states that the difference in IC_{50} values occurs due to differences in DPPH concentration, solvent used and sample growing area.

Table 4. Results of Determination of Antioxidant Activity of Instant Powder of Sungkai Leaves

Extract concentration	Abs control	Abs instant powder + DPPH	Inhibition (%)	IC_{50}
150	0,393	0,382	2,8	662,69
300		0,336	14,50	
450		0,271	31,04	
600		0,217	44,78	
750		0,162	58,78	

Value IC_{50} instant powder of sungkai leaves obtained 662.69 µg/mL. The results of this study showed that the IC_{50} value of sungkai leaf ethanol extract after being made into instant powder increased due to factors from drying at the time of making dry extract and due to the addition of maltodextrin to the instant powder formulation process.

On equatorial research (2020), Explaining that the drying process can cause a decrease in antioxidant activity, the constituent components of antioxidants such as flavonoids and phenolics are easily oxidized. In addition, it may be caused by some potential ingredients as antioxidants oxidized due to a rather long drying process. Therefore, the drying process should not take long so that the chemical content of the material can be maintained from the oxidation process.

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CONCLUSION

Sungkkai leaf ethanol extract can be formulated into instant powder as a health supplement that has met physical requirements. The antioxidant activity test of instant powder preparations of sungkai leaves obtained antioxidant activity with a very weak category.

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